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(54) Title: **MUTANT SODIUM CHANNEL $Na_v1.7$ AND METHODS RELATED THERETO**

(57) Abstract: Described are mutant $Na_v1.7$ sodium channel alpha-subunits and nucleic acid sequences encoding such mutants. Further described are methods for characterizing a nucleic acid sequence that encodes a Na_v1 sodium channel alpha-subunit, methods for determining a $Na_v1.7$ haplotype, methods for determining a subject's predisposition to a neurologic disorder associated with a sodium channel mutation, and methods of identifying a compound that modulates mutant $Na_v1.7$ sodium channels. Other materials, compositions, articles, devices, and methods relating to mutant $Na_v1.7$ sodium channels are also described herein.

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MUTANT SODIUM CHANNEL Na_v1.7 AND METHODS RELATED THERE TO

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims benefit of priority to U.S. Provisional Application No. 60/538,149, filed January 21, 2004. U.S. Provisional Application No. 60/538,149 is incorporated by reference herein in its entirety.

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BACKGROUND

Voltage-gated sodium channels are transmembrane proteins that mediate regenerative inward currents that are responsible for the initial depolarization of action potentials in excitable cells, such as neurons and muscle. Sodium channels are typically a complex of various subunits, the principle one being the alpha-subunit. The alpha-subunit is the pore-forming subunit, and it alone is sufficient for all known sodium channel function. However, in certain sodium channels, smaller, auxiliary subunits called beta-subunits are known to associate with the larger alpha-subunit and are believed to modulate some of the functions of the alpha-subunit. (See Kraner, *et al.* (1985) J Biol Chem 260:6341-6347; Tanaka, *et al.* (1983) J Biol Chem 258:7519-7526; Hartshorne, *et al.* (1984) J Biol Chem 259:1667-1675; Catterall, (1992) Physiol Rev 72:S14-S48; Anderson, *et al.* (1992) Physiol Rev 72:S89-S158.) A review of sodium channels is presented in Catterall, (1995) Ann Rev Biochem 64:493-531.

The primary structures of sodium channel alpha-subunits from a variety of tissues (brain, peripheral nerve, skeletal muscle, and cardiac muscle) and organisms (jellyfish, squid, eel, rat, human) have been identified, and their amino acid sequences show individual regions which have been conserved over a long evolutionary period (see Alberts, *et al.*, eds., "Molecular Biology of the Cell" 534-535, Garland Pub., New York, N.Y. (1994)). From these studies it is known that the alpha-subunit of a sodium channel is a large glycoprotein containing four homologous domains (labeled I-IV in Fig. 1) connected by intracellular loops. The N-terminus of the alpha-subunit extends intracellularly at domain I (*i.e.*, DI) and the C-terminus of the alpha-subunit extends intracellularly at domain IV (*i.e.*, DIV). In the plasma membrane, the four domains orient in such a way as to create a central pore whose structural constituents determine

the selectivity and conductance properties of the sodium channel.

Each domain of the sodium channel alpha-subunit contains six transmembrane alpha-helices or segments (labeled 1-6 in Fig. 1). Five of these transmembrane segments are hydrophobic, whereas one segment is positively charged with several lysine or arginine residues. This highly charged segment is the fourth transmembrane segment in each domain. Extracellular loops connect segment 1 (*i.e.*, S1) to segment 2 (*i.e.*, S2) and segment 3 (*i.e.*, S3) to segment 4 (*i.e.*, S4). Intracellular loops connect S2 to S3 and S4 to segment 5 (*i.e.*, S5). An extracellular re-enterant loop connects S5 to segment 6 (*i.e.*, S6). (See Agnew, *et al.* (1978) Proc Natl Acad Sci USA 75:2606-2610; Agnew, *et al.* (1980) Biochem Biophys Res Comm 92:860-866; Catterall, (1986) Ann Rev Biochem 55:953-985; Catterall, (1992) Physiol Rev 72:S14-S48.)

Voltage-gated sodium channels can be named according to a standardized form of nomenclature outlined in Goldin, *et al.* (2000) Neuron 28:365-368. According to that system, voltage-gated sodium channels are grouped into one family from which nine mammalian isoforms have been identified and expressed. These nine isoforms are given the names Na_v1.1 through Na_v1.9. Also, splice variants of the various isoforms are distinguished by the use of lower case letters following the numbers (*e.g.*, "Na_v1.1a").

Because of the important role sodium channels play in the transmission of action potentials in excitable cells like neurons and muscle, sodium channels have been implicated in many sensory, motor, and neurologic disorders. Accordingly, sodium channels have been the focus of much scientific research. However, while a great deal has been learned about sodium channels, there remains a need for further understanding of the functioning of sodium channels, and means to diagnose, predict, prevent, and treat diseases, disorders, and conditions that result from variations and abnormalities of sodium channels. These and other objects and advantages of the materials, compositions, articles, devices, and methods described herein, as well as additional inventive features, will be apparent from the following disclosure.

SUMMARY

In accordance with the purposes of the disclosed materials, compositions, articles, devices, and methods, as embodied and broadly described herein, the disclosed subject matter, in one aspect, relates to a method of characterizing a nucleic acid sequence that encodes a Na_v1.7 sodium channel alpha-subunit, wherein the method comprises the step of identifying mutations at one or more sites in regions of

the nucleic acid sequence that encode an intracellular *N*-terminal region, an extracellular loop in domain I, an intracellular loop between domains I and II, an intracellular loop between domains II and III, an intramembrane region of domain II, or any combination thereof, such identified nucleotides indicating the character of the nucleic acid sequence.

In another aspect, the disclosed subject matter relates to a method for determining a Na_v1.7 haplotype in a human subject, wherein the method comprises identifying one or more nucleotides encoding amino acid residues 62, 149, 641, 655, 739, 1123, or any combination thereof, wherein the nucleotide or nucleotides indicate the haplotype.

In yet another aspect, the disclosed subject matter relates to a method for determining a subject's predisposition to a neurologic disorder associated with a sodium channel mutation comprising comparing the subject's Na_v1.7 haplotype with one or more reference haplotypes that correlate with the neurologic disorder, a similar haplotype in the subject's Na_v1.7 haplotype as compared to the reference haplotype or haplotypes indicating a predisposition to the neurologic disorder.

In a still further aspect, described herein is a method of identifying a compound that modulates mutant Na_v1.7 sodium channels, wherein the method comprises contacting with a test compound a cell containing a mutant Na_v1.7 nucleic acid that encodes a mutant Na_v1.7 sodium channel comprising one or more mutations at residue 62, residue 149, residue 641, residue 655, residue 739, or residue 1123, detecting Na_v1.7 sodium channel activity, and comparing the Na_v1.7 sodium channel activity in the contacted cell with the amount of Na_v1.7 sodium channel activity in a control cell, wherein the control cell is not contacted by the test compound, an increased or decreased Na_v1.7 sodium channel activity in the test cell as compared to the control cell indicating a compound that modulates mutant Na_v1.7 sodium channels.

Also, described herein are isolated nucleic acids comprising nucleotide sequences encoding mutant Na_v1.7 sodium channel alpha-subunits, expression vectors made from such nucleic acids, cultured cells comprising such vectors, and methods of making mutant Na_v1.7 sodium channel alpha-subunits comprising culturing such cells under conditions allowing expression of the polypeptide encoded by the nucleic acids, wherein the polypeptide comprises a mutant Na_v1.7 sodium channel alpha-subunit. Further, described herein are isolated polypeptides comprising mutant Na_v1.7 sodium

channel alpha-subunits and fragments thereof as well as purified antibodies that bind to epitopes of such mutant Na_v1.7 sodium channel alpha-subunits.

Additional advantages will be set forth in part in the description that follows, and in part will be obvious from the description, or may be learned by practice of the aspects described below. The advantages described below will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive.

10 BRIEF DESCRIPTION OF DRAWINGS

The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several aspects described below.

Figure 1 is a diagram of the secondary structure of a sodium channel alpha-subunit. Not shown is the pore region in each of the four domains, which consists of an inward loop between transmembrane regions 5 and 6.

Figure 2 is a diagram showing the segregation of the N641Y mutation and phenotypic findings of kindred 4425. The following abbreviations are used in the diagram: "fs" means febrile seizures; "afs" means afebrile seizures; "+" means wild type; and "m" means mutant.

Figure 3 is a diagram of the secondary structure of a Na_v1.7 sodium channel alpha-subunit where the locations of various mutations are identified.

Figure 4 is a graph showing current voltage relationships of whole-cell currents. Full-length wild-type SCN9A and mutant SCN9A (K655R and N641Y) constructs were transiently transfected into tsA201 cells. Currents were elicited by test pulses from -60mV to +40mV in 5mV increments. At negative potentials, K655R has a higher current density than wild type. At positive potentials, N641Y has reduced current density compared to wild-type, $p < 0.05$.

DETAILED DESCRIPTION

The materials, compositions, articles, devices, and methods described herein may be understood more readily by reference to the following detailed description of specific aspects of the disclosed subject matter, and methods and the Examples included therein and to the Figures and their previous and following description.

Before the present materials, compositions, articles, devices, and methods are disclosed and described, it is to be understood that the aspects described below are not

limited to specific synthetic methods or specific reagents, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting.

Disclosed herein are materials, compositions, and components that can be used
5 for, can be used in conjunction with, can be used in preparation for, or are products of
the disclosed method and compositions. These and other materials are disclosed
herein, and it is understood that when combinations, subsets, interactions, groups, etc.
of these materials are disclosed that while specific reference of each various
individual and collective combinations and permutation of these compounds may not
10 be explicitly disclosed, each is specifically contemplated and described herein. For
example, if a Na_v1.7 sodium channel is disclosed and a number of modifications that
can be made to a number of amino acid residues or nucleotides, including those
related to the mutant Na_v1.7 sodium channel are discussed, each and every
combination and permutation that are possible are specifically contemplated unless
15 specifically indicated to the contrary. Thus, if a class of substituents A, B, and C are
disclosed as well as a class of substituents D, E, and F and an example of a
combination molecule, A-D is disclosed, then even if each is not individually recited,
each is individually and collectively contemplated. Thus, in this example, each of the
combinations A-E, A-F, B-D, B-E, B-F, C-D, C-E, and C-F are specifically
20 , contemplated and should be considered disclosed from disclosure of A, B, and C; D,
E, and F; and the example combination A-D. Likewise, any subset or combination of
these is also specifically contemplated and disclosed. Thus, for example, the sub-
group of A-E, B-F, and C-E are specifically contemplated and should be considered
disclosed from disclosure of A, B, and C; D, E, and F; and the example combination
25 A-D. This concept applies to all aspects of this disclosure including, but not limited
to, steps in methods of making and using the disclosed compositions. Thus, if there
are a variety of additional steps that can be performed it is understood that each of
these additional steps can be performed with any specific embodiment or combination
of embodiments of the disclosed methods, and that each such combination is
30 specifically contemplated and should be considered disclosed.

Throughout this specification, various publications are referenced. The
disclosures of these publications in their entireties are hereby incorporated by
reference into this application in order to more fully describe the state of the art to
which this pertains. The references disclosed are also individually and specifically

incorporated by reference herein for the material contained in them that is discussed in the sentence in which the reference is relied upon.

Definitions

In this specification and in the claims that follow, reference will be made to a
5 number of terms, which shall be defined to have the following meanings:

As used in the specification and the appended claims, the singular forms “a,”
“an,” and “the” include plural referents unless the context clearly dictates otherwise.
Thus, for example, reference to “a nucleotide” includes mixtures of two or more such
nucleotides, reference to “an amino acid” includes mixtures of two or more such
10 amino acids, reference to “the sodium channel” includes mixtures of two or more such
sodium channels, and the like.

“Optional” or “optionally” means that the subsequently described event or
circumstance can or cannot occur, and that the description includes instances where
the event or circumstance occurs and instances where it does not. For example, the
15 phrase “the array can optionally comprise the most commonly found allele at a .
second...position” means that the most commonly found allele at a second position
may or may not be present in the array and that the description includes both arrays
without the most commonly found allele at the second position and arrays where there
is the most commonly found allele at the second position.

20 Ranges may be expressed herein as from “about” one particular value, and/or
to “about” another particular value. When such a range is expressed, another aspect
includes from the one particular value and/or to the other particular value. Similarly,
when values are expressed as approximations, by use of the antecedent “about,” it will
be understood that the particular value forms another aspect. It will be further
25 understood that the endpoints of each of the ranges are significant both in relation to
the other endpoint, and independently of the other endpoint.

“Subject,” as used herein, means an individual. In one aspect, the subject is a
mammal such as a primate, and, in another aspect, the subject is a human. The term
“subject” also includes domesticated animals (e.g., cats, dogs, etc.), livestock (e.g.,
30 cattle, horses, pigs, sheep, goats, etc.), and laboratory animals (e.g., mouse, rabbit, rat,
guinea pig, etc.).

“Nav1.7,” as used herein, refers to an isoform of a sodium channel known in
the art by names such as NaS, hNE-Na, and PN1. The traditional gene symbol for a
Nav1.7 sodium channel is *SCN9A*, and thus the term Nav1.7, as used herein, is

synonymous with the term *SCN9A*. There are a variety of sequences related to the $\text{Na}_v1.7$ gene having the following Genbank Accession Numbers: NM 002977 (human), U35238 (rabbit), X82835 (human), U79568 (rat), and AF000368 (rat), these nucleic acid sequences, the polypeptides encoded by them, and other nucleic acid and polypeptide sequences are herein incorporated by reference in their entireties as well as for individual subsequences contained therein.

There are a variety of compositions disclosed herein that are amino acid based, including for example $\text{Na}_v1.7$ sodium channel alpha-subunits. Thus, as used herein, "amino acid," means the typically encountered twenty amino acids which make up polypeptides. In addition, it further includes less typical constituents which are both naturally occurring, such as, but not limited to formylmethionine and selenocysteine, analogs of typically found amino acids, and mimetics of amino acids or amino acid functionalities. Non-limiting examples of these and other molecules are discussed herein.

As used herein, the terms "peptide" and "polypeptide" refer to a class of compounds composed of amino acids chemically bound together. Non-limiting examples of these and other molecules are discussed herein. In general, the amino acids are chemically bound together via amide linkages (CONH); however, the amino acids may be bound together by other chemical bonds known in the art. For example, the amino acids may be bound by amine linkages. Peptide as used herein includes oligomers of amino acids and small and large peptides, including polypeptides and proteins.

There are a variety of compositions disclosed herein that are nucleic acid based, including for example the nucleic acids that encode, for example, $\text{Na}_v1.7$ sodium channel alpha-subunits. Thus, as used herein, "nucleic acid" means a molecule made up of, for example, nucleotides, nucleotide analogs, or nucleotide substitutes. Non-limiting examples of these and other molecules are discussed herein. A nucleic acid can be double stranded or single stranded. It is understood that, for example, when a vector is expressed in a cell the expressed mRNA will typically be made up of A, C, G, and U. Likewise, it is understood that if, for example, an antisense molecule is introduced into a cell or cell environment through, for example, exogenous delivery, it is advantageous that the antisense molecule be made up of nucleotide analogs that reduce the degradation of the antisense molecule in the cellular environment.

As used herein, "nucleotide" is a molecule that contains a base moiety, a sugar moiety and a phosphate moiety. Nucleotides can be linked together through their phosphate moieties and sugar moieties creating an internucleoside linkage. The base moiety of a nucleotide can be adenine-9-yl (A), cytosine-1-yl (C), guanine-9-yl (G),
5 uracil-1-yl (U), and thymine-1-yl (T). The sugar moiety of a nucleotide is a ribose or a deoxyribose. The phosphate moiety of a nucleotide is pentavalent phosphate. A non-limiting example of a nucleotide would be 3'-AMP (3'-adenosine monophosphate) or 5'-GMP (5'-guanosine monophosphate).

"Nucleotide analog," as used herein, is a nucleotide which contains some type
10 of modification to either the base, sugar, or phosphate moieties. Modifications to nucleotides are well known in the art and would include for example, 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, and 2-aminoadenine as well as modifications at the sugar or phosphate moieties.

"Nucleotide substitutes," as used herein, are molecules having similar
15 functional properties to nucleotides, but which do not contain a phosphate moiety, such as peptide nucleic acid (PNA). Nucleotide substitutes are molecules that will recognize nucleic acids in a Watson-Crick or Hoogsteen manner, but which are linked together through a moiety other than a phosphate moiety. Nucleotide substitutes are able to conform to a double helix type structure when interacting with the appropriate
20 target nucleic acid.

It is also possible to link other types of molecules (conjugates) to nucleotides or nucleotide analogs to enhance for example, cellular uptake. Conjugates can be chemically linked to the nucleotide or nucleotide analogs. Such conjugates include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger, *et al.*
25 (1989) Proc Natl Acad Sci USA, 86:6553-6556.)

A "Watson-Crick interaction" is at least one interaction with the Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute. The Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute includes the C2, N1, and C6 positions of a purine based nucleotide, nucleotide analog, or nucleotide
30 substitute and the C2, N3, C4 positions of a pyrimidine based nucleotide, nucleotide analog, or nucleotide substitute.

A "Hoogsteen interaction" is the interaction that takes place on the Hoogsteen face of a nucleotide or nucleotide analog, which is exposed in the major groove of duplex DNA. The Hoogsteen face includes the N7 position and reactive groups (NH₂

or O) at the C6 position of purine nucleotides.

“Deletion,” as used herein, refers to a change in an amino acid or nucleotide sequence in which one or more amino acid or nucleotide residues, respectively, are absent relative to the reference sequence.

5 “Insertion” or “addition,” as used herein, refers to a change in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid or nucleotide residues, respectively, as compared to the reference sequence.

 “Substitution,” as used herein, refers to the replacement of one or more amino acids or nucleotides by one or more different amino acids or nucleotides, respectively,
10 in a reference sequence.

 “Isolated,” as used herein refers to material, such as a nucleic acid or a polypeptide, which is: (1) substantially or essentially free from components which normally accompany or interact with it as found in its naturally occurring environment. Although, the isolated material optionally comprises material not found
15 with the material in its natural environment; or (2) if the material is in its natural environment, the material has been synthetically (non-naturally) altered by deliberate human intervention to a composition and/or placed at a locus in the cell (*e.g.*, genome or subcellular organelle) not native to a material found in that environment. The alteration to yield the synthetic material can be performed on the material within or
20 removed from its natural state.

Characterizing Mutant Na_v1.7 Nucleic Acid Sequences

 It has been found that, in certain neurologic disorders, specific sites in the Na_v1.7 gene are mutated, *i.e.*, the nucleotide at a specific position or at specific positions differs from that observed in the most commonly found Na_v1.7 gene
25 sequence. Accordingly, disclosed herein are methods of characterizing mutant nucleic acid sequences that encode a Na_v1.7 sodium channel alpha-subunit and the use of such nucleic acids to diagnose and treat disease states and neurologic disorders, such as seizures.

 In one aspect, disclosed herein is a method of characterizing a nucleic acid
30 sequence that encodes a Na_v1.7 sodium channel alpha-subunit, comprising the step of identifying mutations at one or more sites in regions of the nucleic acid sequence that